such that amino acids are produced by said microbial organism with increased efficiency.

3. A process according to claim 2, wherein an export carrier with increased export activity is generated by mutation of the endogenous export gene.

4. A process according to claim 1, wherein the export gene expression of the export carrier is increased by increasing the number of gene copies.

13. A process according to one of claims 10 to 12, wherein, for the transformation, a microorganism is utilized which contains an increased amount of the metabolites of the central metabolism.

REMARKS

The Examiner has rejected claims 8 - 10 and 16 - 18 under 35 USC 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected to make and/or use the invention.

With regard to the Examiner's reasons for this rejection as presented on page 3 and the first two lines of page 4 of the Official Action, it is noted that under the term "allelic variations" various configurations of genes (nucleotide sequences) are to be understood which are the result of the degeneration of the genetic code, or , respectively, of function-neutral mutations of the nucleotide sequence (deletion, insertion, substitution). A change of the nucleotide sequence may be noticeable phenotypically in that the protein coded by the allel (the gene variation has a changed (for example, increased or reduced) specific activity. It is however noted that the function of the coded protein remains basically the same that is the specific export carrier activity remains a specific export carrier activity even if the respective protein is coded by a somewhat different ("changed" or "allel") nucleotide sequence. Merely the level of the activity is changed, preferably increased.

Besides variations in the coded area of a nucleotide sequence, a nucleotide sequence may vary also in areas, which are responsible for the regulation of the gene expression, so that the allele nucleotide sequences result, for example, in a stronger gene expression and consequently in an increased number of copies of the coded proteins.

It is pointed out that the expression "allelic variations" is a technical term, which is well known to any expert in the field of genetics. As an example for a standard reference book, "Molecular Biology of the Cell", Alberts et al. is mentioned.

Furthermore, procedures, by which characteristic coding or non-coding (upstream) of a nucleotide sequence can be identified whose changes result in gene alleles, which finally code for gene products with the desired advantageous properties, are part of the common laboratory praxis of any technician or expert in the field (Sambrook et al., Molecular Cloning, A Laboratory Manual, 1989). Such characteristic areas are, for example, the catalytic or regulatory center or transmembrane areas in the coding section of a nucleotide sequence or regulatory areas (for example promoter, enhancer, terminator or ribosome bonding locations) upstream of the coding area of a nucleotide sequence.

Changes may occur, for example, by known processes such as natural, classical, chemical, or gene technical mutation. An allele of the nucleotide sequence then codes for example a protein with an increased expert carrier activity. See in this respect page 5, line 6 - page 6, second paragraph of the description. Instructions are also found ao in the manual Sambrook et al. Molecular Cloning - A Laboratory Manual - 1998.

Consequently, the person skilled in the particular art is quite knowledgeable on how to obtain alleles of a nuclide sequence and how to utilize advantageous variants and the proteins coded thereby. Reconsideration of the Examiner's rejection of claims 8 - 10 and 16 - 18 under 35 USC 112, first paragraph is respectfully requested.

As to the Examiner's rejection of claims 1 - 20, 44 and 45 under 35USC 112, second paragraph, claim 1 has been amended so as to define the invention more clearly.

The Examiner's rejection of those claims as failing to particularly point out and distinctly claim the subject matter considered to be the invention is not considered to be proper.

It is noted that the steps considered by the Examiner to be missing from claim 1 are all specific steps which, in Applicant's opinion are not necessary to be defined in claim 1 in the details as proposed by the Examiner. They are specifically defined in subclaims.

The particular steps and techniques for increasing the expression of a gene and for increasing the activity of a carrier protein, which provides for the export of an amino acid the specific production of which is simplified by a microorganism and the techniques for increasing the expression of a gene that codes for a specific amino export carriers are part of usual laboratory practices and are well-known to the person skilled in the art. some of the possibilities are described for example on page 5, line 7 to page 6, line 19.

The steps omitted from claim 1 in the view of the Examiner represent only a particular one of several possible specific variation in a method for the microbacterial manufacture of amino acids. Those specific steps are covered in the subclaims.

In principle in accordance with the present invention, an export carrier protein, which is specific for an amino acid, (such specific export carrier proteins are not known from the state of the art) and a respective coding gene are provided whereby, as a result, the activity of the amino acid export carrier and the expression of the coding gene is increased resulting in a substantial improvement of the microbacterial production of amino acids.

The reason for the advantageous result is that a wild-type bacteria cell has only a limited capability to discharge the amino acids formed within the cell. This occurs by way of a non-specific mechanism such as by diffusion, by way of pores or non-specific membrane proteins. Such non-specific transport mechanisms however are substantially less effective than specific transport mechanisms in discharging a respective amino acid actively out of the cell.

This problem of the limited capability of discharging amino acids from a bacteria cell is an important handicap for the microbacterial production of amino acids. The problem is solved by the present invention in that the endogenous activity of the export carrier protein specific for a respective amino acid is increased and the endogenous expression of the gene coding for this carrier protein are also increased.

In an exemplary embodiment of the present invention, the gene for the export carrier was isolated from the bacteria strain C. glutamicum R127 (see description page 9, example a). This bacteria strain is a C. glutamicum wild-type, which is merely restriction-negative; that is, it breaks up unspecific DNA to a lesser degree and is therefore very suitable for cloning activities. Concerning its biosynthesis paths and, in the present instance, specifically its amino acid biosynthesis paths, this strain R127 however is not changed. The isolated DNA and the gene bank prepared therefrom corresponds accordingly to the C. glutamicum wild-type DNA.

This DNA gene bank was subsequently transformed into the C. glutamicum strain NA8. The strain comprises also the C. glutamicum wild-type derivative R127, which has no longer a natural amino acid excretion and which is designated therefore a "non-excreter".

Also, this strain C. glutamicum NA8 is not changed with respect to its amino acid biosynthesis and therefore corresponds to the C. glutamicum "wild-type". It is therefore not an especially produced amino acid production strain.

As a result, a C. glutamicum "wild-type" DNA gene bank was converted to a C. glutamicum "wild-type". The DNA clone from the gene DNA bank, which forms from the "wild-type" strain NA8 ("non-excreter") again an amino acid excreter, contained the export carrier gene according to the invention. The amino acid excretion was demonstrated by means of so-called indicator agar plates, whose manufacture is described on page 10 of the description.

With the procedure described up to this point, the export carrier according to the invention specific for amino acids is isolated. The export carrier was subsequently genetically and biochemically analyzed and characterized.

Finally - and this is essential for the present invention - the activity of the non-identified export carrier in the C. glutamicum strain NA8, which is not an amino acid production strain, but corresponds, with regard to amino acid biosynthesis to the wild-type of C. glutamicum, is increased (page 13, example e).

This increase in the activity of the export carrier specific for amino acids resulted in an improved amino acid excretion into the surrounding culture medium and, consequently, in an improved amino acid production.

Consequently, it is solely the increased export carrier activity, which provides for the improved amino acid production.

The present invention therefore is an enormous step in the development in this field. It would not be justifiable to punish the inventor for the disclosure of the invention by requiring a limitation of claim 1 to an increased gene expression (by producing a gene construct, transformation of the construct etc... as proposed by the Examiner). It is to be understood that an increased gene expression can be achieved in various ways known to the person skilled in the art. On the other hand, also an increase in the protein activity of the export carrier can, in accordance with the invention, result in an improved amino acid production. The person skilled in the art is aware of various ways (see also the description pages 5 - 7).

In any case however, it has been shown that alone the increase of the specific export carrier activity in "wild-type" bacteria results in a substantially increased yield in the amino acid production.

A limitation of claim 1 in this respect is therefore not necessary and such a requirement is not justifiable.

As pointed out earlier, the person skilled in the art knows the procedures required for increasing an endogenous protein activity and/or the gene expression and can easily select one that is suitable for the particular circumstances. A particular selection is disclosed on page 5, line 7 to page 6, line 19 of the present application (substitute copy).

With regard to the Examiner's objections to claim 3, it is noted that according to claim 3, an export carrier with greater export activity is generated by mutation of the endogenous export gene. Possible mutations are described on page 5, second paragraph.

Mutations of the export gene are degenerations of the genetic code resulting from function-neutral sense mutations or mutations in regulatory non-coding areas of a nucleotide sequence. They are known to the person skilled in the art as pointed out earlier with regard to the allelic variations. In order to achieve an increased export carrier activity, the

person skilled in the art may change for example the regulatory areas of the non-coding nucleotide sequence such as the promoter, the enhancer or ribosome bonding locations. Advice in this regard is found for example in the standard manual Sambrook et al., "Molecular Cloning: A Laboratory Manual", 1998.

It is hoped that the above comments overcome the Examiner's concerns regarding the "mutants" as contained in claims 3 and 44.

Reconsideration of these claims is respectfully requested.

In claim 13, the expression "central metabolism metabolites", which was considered by the Examiner to be indefinite, has been changed.

Reconsideration is respectfully requested.

With regard to the Examiner's rejection of the claims under 35 USC 102 in view of the inventors publication in "Molecular Microbiology", Vol. 22(5), pp. 815-826, 1996, certified copies of the original German and of the PCT application are enclosed. Both have the identical text. A certified translation of German priority document is also enclosed in order to establish the priority date of 12/22/95.

Reconsideration of the rejection of the claims under 35 USC § 102 is respectfully requested.

Allowance of claims 1-20 and 43-48 is solicited.

Respectfully submitted,

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MARK-UP VERSION TO SHOW CHANGES MADE

1. A process for the microbacterial production of amino acids, [wherein the] comprising the steps of:

providing a mcirobial organism having a certain export carrier activity and [the] a certain export gene-expression, [of a microorganism producing the respective amino acid is increased]

increasing, selectively, one of

the export carrier activity of said microbial organism specific for a particular amino acid with an amino acid sequence as given in SEQ ID No. (A)2 and in table 3, including any allele variation thereof, in accordance with the export carrier activity endogenous to said microbial organism,

and the export gene expression of said microbial organism specific for a particular amino acid with a nucleotide sequence of nucleotide 1016 to 1726 according to SEQ ID No. (A)1 and table 2, or a DNA sequence with essentially the same effects, in accordance with the export gene expression endogenous to said microbial organism by means of one of the steps selected from the group of:

i) mutating the export carrier gene, such that an export carrier with increased export activity is generated,

ii) increasing the number of gene copies of the export carrier gene,

iii) modifying regulatory signals assigned to the export gene, and

iv) amplifying regulatory signals assigned to the export gene,

such that amino acids are produced by said microbial organism with increased efficiency.

3. A process according to claim 2, wherein[, by mutation of the endogenous export gene, a] an export carrier with [higher] increased export activity is generated by mutation of the endogenous export gene.

4. A process according to [one of the claims 1 to 3] claim 1, wherein the export gene expression of the export carrier is increased by increasing the number of gene copies.

13. A process according to one of claims 10 to 12, wherein, for the transformation, a microorganism is utilized which contains an increased [part] amount of the metabolites of the central metabolism [metabolites].